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Anti-inflammatory and antitumour activities of cultured mycelium of morel mushroom, *Morchella esculenta*

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Mushrooms are nutritionally functional food and a source of physiologically beneficial and non-toxic medicines. They have been used in folk medicine throughout the world since ancient times. *Morchella esculenta* (L) Pers. is an edible and highly priced mushroom. Commercial cultivation of this mushroom has not been successful till now and hence its mycelium is extensively used as a flavouring agent. Anti-inflammatory and antitumour activities of ethanolic extract of cultured mycelium of *M. esculenta* were investigated. The extract showed significant dose-dependent inhibition of both acute and chronic inflammation. The activity was comparable to that of the standard reference drug, Diclofenac. Antitumour activity of the extract was determined using both DLA cell line-induced solid tumour and EAC cell line-induced ascites tumour models in mice. The extract exhibited significant antitumour activity against both ascites and solid tumours. The finding suggests the potential therapeutic use of aqueous-ethanolic extract of morel mushroom mycelium in chemotherapy.

Keywords: Anti-inflammatory activity, antitumour activity, cultured mycelium, medicinal mushrooms, *Morchella esculenta*.

INFLAMMATION, a fundamental protective response, can be harmful in conditions such as life-threatening hyper-

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sensitive reactions to insect bites, drugs, toxins and in chronic diseases such as rheumatic arthritis, atherosclerosis, lung fibrosis and cancer[1]. Inflammation can also accelerate cancer and chronic inflammation is regarded as an essential factor for the progression of the neoplastic process[2].

Cancer is one of the leading causes for human death[3]. In modern medicine, chemotherapy, radiotherapy and surgery are the major modes of cancer treatment[4]. Intervention with chemopreventive agents in the early stage of carcinogenesis is theoretically more rational than attempting to eradicate fully developed tumours with chemotherapeutic drugs[5]. These agents have a narrow margin of safety, and the therapy may fail due to drug resistance and dose-limiting toxicities, which may severely affect the host normal cells[3]. Hence the use of natural products has been contemplated in the control of cancer and its eradication programme[6].

Mushrooms are nutritionally functional foods and a source of physiologically beneficial and noninvasive medicines. Many pharmaceutical substances with potent and unique health-enhancing properties have been isolated from medicinal mushrooms and distributed worldwide. Mushroom-based products either from the mycelia or fruiting bodies are consumed in the form of capsules, tablets or extracts. Some of the most recently isolated and identified substances from mushrooms have been demonstrated to possess significant antitumour, cardiovascular, antiviral, antibacterial, antiparasitic, hepatoprotective and antidiabetic activities[7].

Morels are one of the most highly priced mushrooms found in the world. *Morchella esculenta* (L) Pers. is an edible morel mushroom. In India, this mushroom is found growing in the forests of Jammu and Kashmir, and Himachal Pradesh. Morels are locally known as Guchhi and are used in healthcare as well as for medicinal purposes by traditional hill societies[8,9]. Since commercial cultivation of morels for the fruiting bodies has not been successful till now, the cultured mycelium is extensively used as a flavouring agent. Proteins from the mycelia of *Morchella* are comparable to vegetative protein and can be used as a good source of protein supplement[10]. Approximately 80% of mushroom products is produced from the fruiting bodies[11]. Cultivation of mushroom for fruiting-body production is a long-term process taking one to several months depending upon the species and substrates. In contrast, production of mushroom mycelium in submerged culture would allow acceleration in the growth and to obtain high yield of biomass with constant composition. Hence cultured mycelium of mushrooms is an ideal source for developing healthcare products. In this communication, we report the anti-inflammatory and antitumour activities of the ethanolic extract of cultured mycelium of *M. esculenta*.

A culture of *M. esculenta* (MTCC 1795), obtained from Microbial Type Culture Collection, Institute of Microbiology, Chandigarh was used for the study. The fungus was grown in submerged culture on Potato Dextrose Broth (PDB) for the production of mycelial biomass. After 10 days of growth at 24–25°C in submerged culture[10], the fungal biomass was harvested, washed thoroughly and dried at 40–50°C.

The dried mycelia were powdered and 100 g powder was extracted with hot aqueous-ethyl alcohol (ethyl alcohol : water 50/50 v/v) for 8–10 h. The extract was concentrated and solvent completely evaporated under vacuum. The residue (6%) thus obtained was employed for the experiments.

Female Swiss albino mice were purchased from Small Animal Breeding Centre, Veterinary College, Thrissur, Kerala. They were kept for a week under environmentally controlled conditions with free access to standard food (Sai Durga Feeds, Bangalore) and water *ad libitum*. Mice weighing 25 ± 2 g were used for the study. All animal experiments were carried out according to the guidelines and approval of the Animal Ethics Committee.

Dalton's lymphoma ascites (DLA) and Ehrlich's ascites carcinoma (EAC) cell lines were obtained from Cancer Institute, Adayar, Chennai. The cells were maintained at our centre by intraperitoneal inoculation of 1×10^6 viable cells in mice.

For carrageenan-induced paw oedema, the animals were divided into four groups of six animals each. Acute inflammation was produced in all animals by subplantar injection of 20 µl freshly prepared 1% suspension of carrageenan in normal saline on the right hind paw of mice[12]. Paw thickness was measured using a vernier calipers before and after carrageenan challenge in each group. Animals were premedicated with extract (250 and 500 mg/kg body wt) and the reference drug, Diclofenac (10 mg/kg body wt), orally 1 h before carrageenan injection.

For dextran-induced paw oedema the animals were treated as in the case of carrageenan-induced paw oedema model, except that in place of carrageenan, dextran was used to induce inflammation[13].

For formalin-induced paw oedema the animals were treated in the same way as in the above models, except that formalin (20 µl of freshly prepared 2% formalin) was used as the oedematogenic agent. The drug treatment continued for six consecutive days[14]. Diclofenac (10 mg/kg body wt) was used as the reference drug.

In all the above models, the degree of oedema formation was determined as increase in paw thickness. Increase in paw thickness and per cent inhibition were calculated as follows. Increase in paw thickness in control/treatment $P_C/P_T = P_t - P_0$.

Per cent inhibition = $(P_C - P_T \times 100)/P_C$, where P_t is paw thickness at time t, P_0 is initial paw thickness, P_C is increase in paw thickness of the control group and P_T is the increase in paw thickness of the treatment groups[15].

Antitumour activity of the extract was determined using ascites and solid tumour models.

In the case of the ascites tumour model, the animals were divided into five groups of six animals each. All the

animals were injected intraperitonially (i.p.) with 1×10^6 viable EAC cells in PBS (aspirated from 15-day-old EAC ascites tumour in mice). After 24 h of tumour cell inoculation, the extract was administered orally at a dose of 250, 500 or 1000 mg/kg body weight and continued for ten consecutive days. The group that received only the cell lines served as control. Cisplatin (4 mg/kg body wt, i.p.) was used as the standard reference drug. The mortality rate was noted in each group and the per cent increase in lifespan (ILS) was calculated using formula % ILS = $(1 - T/C)$, where T is the mean survival time of the treated group and C that of the control group[16].

The effect of the extract when administered simultaneous with tumour inoculation (preventive effect) was determined. Animals were divided into five groups of six animals each. Viable DLA cells 1×10^6 in 0.1 ml PBS were transplanted subcutaneously into the right groin of mice. Ethanolic extract of the mycelium (250, 500 or 1000 mg/kg body wt) were administered orally 24 h after tumour implantation and continued for ten consecutive days. The control group received only the cell line. Cisplatin (4 mg/kg body wt i.p.) was used as the standard reference. Tumour development in the animals of each group was determined by measuring the diameter of tumour growth in two perpendicular planes using vernier calipers twice a week for five weeks. The tumour volume was calculated using the formula $4/3\pi r_1^2 r_2$, where r_1 is the minor diameter and r_2 the major diameter[17]. At the end of the fifth week, animals were sacrificed under anesthesia using diethyl ether, the tumour extirpated and weighed. Per cent inhibition was calculated using the formula $(1 - B/A) \times 100$, where A is the average tumour weight of the control group and B that of the treated group[17].

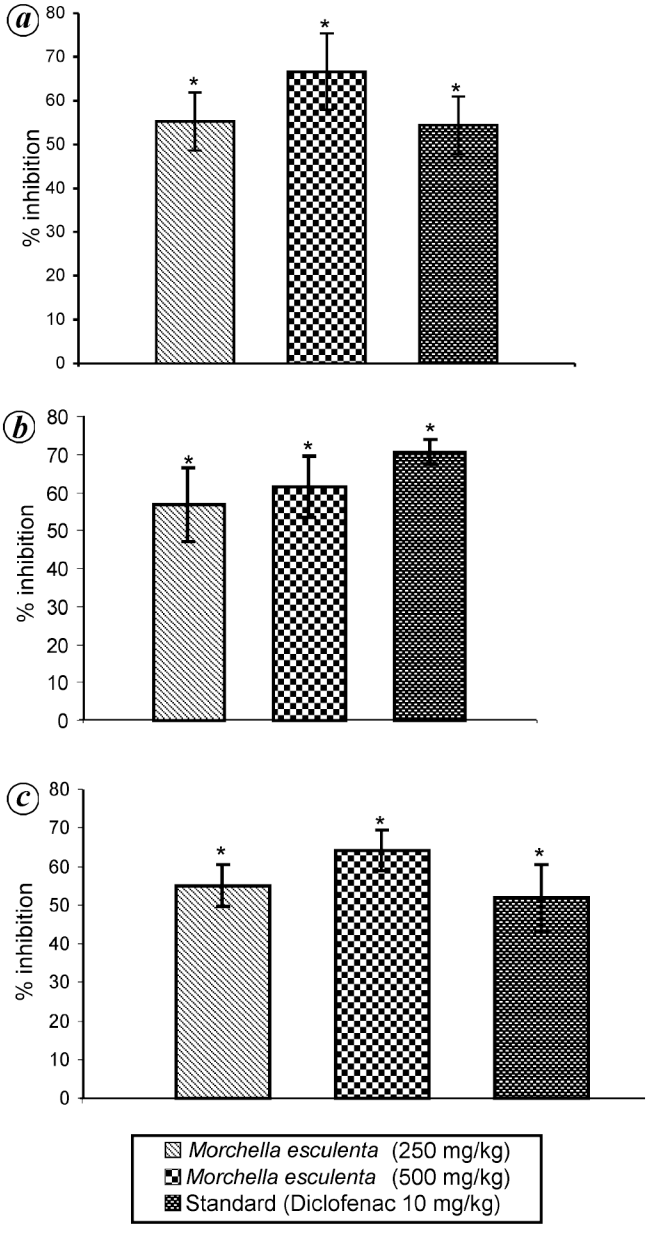
The effect of the extract when administered after tumour development (curative effect) was determined. For this antitumour activity of the extract was tested on tumour-bearing mice. Solid tumour development in mice was induced as described earlier. After 15 days, animals with tumour size around 1.1 ± 0.1 cubic cm were divided into five groups of six animals each. The extract (250, 500 or 1000 mg/kg body wt, p.o.) was administered for ten consecutive days. The group that received only the cell lines served as control. Tumour diameter was measured using a vernier calipers twice a week for a period of three weeks after drug administration, and the volume was calculated[17]. At the end of the fifth week, the animals were sacrificed, the tumour extirpated and weighed. Percentage inhibition was calculated as described earlier[17].

The data were statistically analysed using Student's t test and P values less than 0.001 were considered significant. All data were represented as mean ± SD.

The ethanolic extract of *M. esculenta* mycelium significantly inhibited acute inflammation induced by carrageenan and dextran and chronic inflammation induced by formalin at concentrations of 250 and 500 mg/kg body wt in experimental animals in a dose-dependent manner ($P < 0.001$; Figure 1 *a–c*). The extract at a concentration of 500 mg/kg body wt showed higher activity than the reference drug, Diclofenac, in carrageenan and formalin-induced inflammations.

In the ascites tumour model, the extract at a dose of 1000 mg/kg body wt increased the lifespan of animals by 54.9% ($P < 0.001$; Table 1). The standard reference drug (Cisplatin 4 mg/kg, i.p.) exhibited 61.15% ILS ($P < 0.001$).

The extract also possessed significant antitumour activity against solid tumour models. The extract when admin-



All values are mean ± SD ($n = 6$), *$P < 0.001$ with respect to control

Figure 1. Effect of aqueous-ethanolic extract of *Morchella esculenta* mycelium on (*a*), carrageenan-induced acute inflammation; (*b*), dextran-induced acute inflammation; (*c*), formalin-induced chronic inflammation.

Table 1. Effect of aqueous-ethanolic extract of *Morchella esculenta* mycelium on increase in lifespan of ascites tumour-bearing animals

Group	Treatment (mg/kg)	Survival time (days)	% increase in lifespan	Mortality at 40th day
Control	–	24.30 ± 3.40	–	6/6
Standard	4	34.10 ± 2.40***	61.15	1/6
M. esculenta				
	250	33.80 ± 7.46*	39.09	3/6
	500	34.80 ± 8.06*	43.20	2/6
	1000	37.66 ± 5.70***	54.90	1/6

All values are mean ± SD ($n = 6$); ***$P < 0.001$; *$P < 0.01$ with respect to control.

Table 2. Effect of aqueous-ethanolic extract of *M. esculenta* mycelium on solid tumour (preventive effect)

Treatment (mg/kg)	Tumour volume (cubic cm)	% decrease in tumour volume	Tumour weight	% Decrease in tumour weight
Control	5.490 ± 1.080	–	4.380 ± 0.36	–
Standard (Cisplatin 4 mg)	0.805 ± 0.268***	85.4	0.916 ± 0.110***	79.1
M. esculenta				
250	2.700 ± 0.270***	47.8	2.583 ± 0.480***	41.1
500	2.220 ± 0.590***	59.6	1.680 ± 0.270***	61.7
1000	1.390 ± 0.280***	74.7	1.016 ± 0.210***	76.9

All values are mean ± SD ($n = 6$); ***$P < 0.001$ with respect to control.

istered 24 h after tumour implantation at doses of 250, 500, 1000 mg/kg body wt, prevented 47.87, 59.6 and 74.7% of solid tumour volume and 41.1, 61.7 and 76.9% of tumour weight respectively (Table 2). The weight and volume of the tumour in the extract-treated groups of animals were significantly lower than the control group ($P < 0.001$). The higher concentration of the extract (1000 mg/kg) inhibited tumour proliferation as effectively as the standard reference drug, Cisplatin.

The extract was also found to be highly effective against developed solid tumour. Treatment with the extract at doses of 1000, 500 and 250 mg/kg body wt for ten consecutive days after tumour development, showed 75.30, 65.25 and 59% of tumour volume and 76.5, 65.0 and 52.0% of tumour weight regression respectively, compared to the control (Table 3).

There is significant interest in the use of mushrooms and/or mushroom extracts as dietary supplements based on theories that they enhance immune function and promote health[18]. Extracts of many mushrooms used in traditional Chinese medicine and other folk medicine have been reported to be efficacious in the treatment of various diseases, including many forms of cancer. The use of medicinal mushroom extracts against cancer is well documented in China, Japan, Korea, Russia and now increasingly in USA[19].

The results of the present investigation indicate that the ethanolic extract of *M. esculenta* shows profound anti-inflammatory activity. Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. Development of carrageenan-induced oedema is biphasic; the first phase is attributed to the release of histamie, 5-HT and kinins, while second phase is related to the release of prostaglandins[20–22]. It has been reported that the second-phase oedema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents[23]. Dextran-induced paw oedema is known to be mediated both by histamine and serotonin. Carrageenan and dextran induce paw oedema by different mechanisms. Dextran induces fluid accumulation because of mast cell degranulation with little protein and few neutrophils. Carrageenan induces a protein-rich exudate containing a large number of neutrophils[24].

Formalin-induced paw oedema is one of the most suitable test procedures to screen chronic anti-inflammatory agents, as it closely resembled human arthritis[25]. The nociceptive effect of formalin is also biphasic; an early neurogenic component followed by a later tissue-mediated response[26]. The result suggests the usefulness of *M. esculenta* extract in the treatment of inflammation-associated diseases like arthritis.

The ethanolic extract of *M. esculenta* mycelium is also found to possess significant antitumour activity against both ascites and solid tumour. The results indicate that the extract possessed both curative and preventive properties against solid tumour in a dose-dependent manner. The extract is also significantly effective against ascites tumour. These results suggest that *M. esculenta* mycelia contain compounds that may modulate tumourigenesis at different stages or may act at the same stage. Polysaccharide isolated from the fruiting bodies of *M. esculenta* has been reported to exhibit immunostimulatory activity[27]. Hence,

Table 3. Effect of aqueous-ethanolic extract of *M. esculenta* mycelium on solid tumour (curative effect)

Treatment (mg/kg)	Tumour volume (cubic cm)	% decrease in tumour volume	Tumour weight	% decrease in weight
Control	3.080 ± 1.650	–	5.300 ± 2.500	–
Standard (Cisplatin 4 mg)	0.895 ± 0.539*	70.94	1.460 ± 0.570***	73.0
M. esculenta				
250	1.260 ± 0.600	59.00	2.550 ± 0.790	52.0
500	1.077 ± 0.550	65.25	1.860 ± 0.580**	65.0
1000	0.759 ± 0.200**	75.30	1.200 ± 0.360***	76.5

All values are mean ± SD ($n = 6$); ***$P < 0.001$, **$P < 0.005$, *$P < 0.01$ with respect to control.

morel mushroom mycelium extract possibly provides additive or even synergistic effect in the prevention and treatment of cancer. The findings suggest the potential therapeutic use of morel mushroom mycelium in chemotherapy.

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